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ORIGINAL ARTICLES

THE PROTEOLYTIC ACTIVITY OF NON-PATHOGENIC AND PATHOGENIC FUNGI

By

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PART I

Non-pathogenic Fungi

It has long been known that certain fungi grow on hairs, often causing them to fracture. The observation that these fungi, the dermatophytes, will penetrate hairs *in vitro* gives no indication of the chemical processes involved, but enzyme activity has long been recognized as a likely explanation. Such proteolytic enzymes which might be responsible have not so far been well characterised.

Although considerable work has been done on the proteolytic enzymes of bacteria and protozoa, there has been comparatively little work carried out on those of fungi, particularly the pathogenic fungi. The proteolytic enzymes of fungi appear to have modes of action which differ materially from those of bacteria. Bainbridge (1911) recorded the observation of Martin that when flasks of pure albumin solution were exposed in the laboratory no bacterial growth occured, though there were often good growths of contaminating fungi present.

Hansen (1899) found that *Penicillin glaucum* (or its glycerol extract) liquified gelatin, particularly in neutral solution. Bourquelot (1893) (a) (b), 1894, 1897) grew *Aspergillus niger* on Raulin's medium, obtained an extract by grinding the culture in chloroform-water with sand, and showed that this would digest egg white liberating peptones. Since the action took place in weak acid solution it was presumed to be tryptic in nature. Malfitano (1900) grew *A. niger* on the same medium and distinguished between "Le proteolyse" which attacked true proteins and "La diastase proteolytique" which liquified gelatin. He thought that the composition of the medium did not affect enzyme production so long as adequate growth of fungus took place.

Maze (1905) found that *P. candidum* and *P. glaucum* rapidly digested the caseinogen of milk. Abderhalden and Pringsheim (1909) observed the action of *Allescheria gayonii, Rhizopus tonkineinsis, Aspergilus wentii* and *Mucor mucedo* on various substrates. They demonstrated that not only naturally-occuring d-alanine

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was attacked, but also l-alanine, which does not occur in nature. Later, considerable impetus was given to the study of enzymes of fungi from the elucidation of the part they play in the ripening of cheese (Haines, 1934).

Dox (1909), working with *Penicillium cameberti*, showed that tne production of enzymes normally formed by the organism can be enhanced by special methods of cultivation. The fungus was grown for 10 days on a medium containing sodium nitrate, cane sugar, potassium phosphate and chloride, and ferrous and magnesium sulphates. The mycelium produced was washed in running water, homogenised, treated with acetone and ether and finally dried and powdered. Dox believed that the enzyme present in the extract was closely related to erepsin. It did not attack native proteins such as fibrin and ovalbumin, nor did it break down casein, gelatin nor proteoses. It was most active in neutral or faintly acid medium. Reed and Stahl (1911) also showed that *Glomerella rumonaculaus* and *Sphaerophis malorum* produce an ereptic enzyme in Dunham's solution. It liquified gelatin and converted peptone into tryptophane. Scales (1914) extracted the dried mycelium of *A. terricola*, according to Dox's method, and found that this enzyme attacked coagulated albumin, peptone, gelatin and milk.

Waksman (1918) used Czapek's medium with either peptone or sodium nitrate as the source of nitrogen for producing fungal enzymes. He reached the conclusion that such enzymes had a greater range of optimum activity and a lower temperature optimum than had animal proteases. They were not precipitated by safranin, as was animal trypsin, and would pass through Pasteur-Chamberland candles. Enzymes obtained from protein-free medium cultures were not as active as those from protein -containing media The exo-and endo-enzymes of the fungi he studied decomposed fibrin, crystaline egg, casein and peptone. Johnson (1934) showed that at least four protein-hydrolysing compounds could be extracted from the mycelia of *A. parasiticans*. At about the same time, Burger *et al* (1936) studied the proteolytic enzyme content of 30 common moulds and found that aspergilli were, in general, more potent producers of enzymes than were penicillia. Crewther & Lennox (1953) working on *A. oryzae* described the order of appearance of various enzymes on a protein-free sucrose-tartrate medium. In 1953, McConnell and co-workers showed that the enzymes obtained from submerged cultures of fungi would hydrolyse synthetic dipeptides and their derivatives.

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THE PROTEOLYTIC ACTIVITY OF NON PATHOGENIC AND PATHOGENIC FUNGI

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PART II

Pathogenic fungi

(Dermatophytes)

Verujsky (1887) was the first to initiate studies of the physiology of ringworm fungi in his work with Trichophyton tonsurans. In 1894 Roberts inoculated an "artificially-reared" Trichophyton species on to human and animal hairs which had been removed. He observed that the fungus penetrated and disintegrated much as occurs in the natural process of ringworm infection. For the first time he was impressed that the process of disintegration was likely to be essentially one of digestion. Thereafter, there has been a continuing interest in the proteolytic activity of the metabolic products of pathogenic fungi.

Macfadyen (1894) working with some strains of ringworm fungi (species not named) drew conclusions from findings using different "soils" for growing them. He found that the solution of gelatin in which the fungi were grown possessed an active proteolytic property, and that such activity could be destroyed by heating for two minutes at 100°C. Acid reaction retarded this enzyme activity, while alkali enhanced it. He was not, however, able to satisfy himself that the proteolytic substance had any action on fibrin or hair, in spite of the fact that his Trichophyton strains would grow on " soils" composed almost entirely of keratin.

Roberts (1899) attempted to show that Trichophyton cultures in dried form would decompose gelatin by enzyme action. He concluded that "amylotic ferment" was not produced by the trichophyta and "Trichophyton microsporon" directs its keratolytic powers first at the cuticle and later to deeper keratin of hair, while "Trichophyton megalosporon" acts mainly by digesting the inner substance of hair leaving a relatively unaffected hollow shell of cuticle. He also emphasised that the enzymes he described, and which he called 'keratolytic', were different from the 'proteolytic' ferments previously described by Macfadyen. Roberts went on to criticise Sabouraud's division of trichophyta into "ectothrix" and "endothrix" types (Sabouraud, 1910) and suggested a reclassification according to their enzyme production.

Tate (1929) concluded from his studies that proteolytic enzymes are present in the dermatophytes which possessed properties very similar to trypsin. Using congo red fibrin he did not find any evidence that trypsin was amongst them. He also found that these fungi could readily split tributyrin into fatty acids, presumably due to the presence of a lipase. His results suggested that urease and amygdalin were present in greater amouut in pleomorphic forms than in normal cultures. He did not think that pleomorphism per se changed the enzymatic activities of these fungi.

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Verujsky's (1887) comparative studies of the morphology and physiology of T. tonsurans and Achorion schoeleinii was the earliest work on the physiology of dermatophytes. He found that a neutral or slightly acid medium was the most favourable for growth of the fungi and that the optimum temperature was about 33°C; he found that both fungi would liquify gelatin. Achorion schoenleinii was found to assimiliate sugar in any form, while T. tonsurans utilized glucose but not saccharose. On malt medium he found that the ratio of "weight of Trichophyton to sugar consumed" is 1 : 2 but when if glycerin was added to the ' cospora ' burm of microsporon of the medium this ratio was increased to 2 : 3.

Bodin (1899) found that the cospora form of "microsporon of the horse" (M. equinum) had an optimum temperature for growth of about 35°C. Glucose, dextrin and maltose were assimilated in this order of preference, but sucrose was not utilized. Later, he and Lenormaud (1901) showed that this fungus produces two enzymes in the culture fiuid, one which clots the milk and the other which dissolves the clot.

Tate (1929) studied a number of dermatophytes for their enzyme contents, but his results were inconclusive. More recently, Bentley (1953) and Chattaway et al (1954) demonstrated amino acid oxidase and asparaginase activity in ringworm fungi. In 1956, Cruickshank and his colleagues were able to demonstrate the skin-splitting property of filtrates of T. mentagrophytes.

Barlow and Chattaway (1955) studied the effect on susceptibility to fungal attack of changing the molecular structure of hair keratin. They showed that measures which encourage the breakdown of disulphide linkages and hydrogen bonds facilitate fungal invasion in vitro. The reverse is the case when cross-linkages are increased between free amino and carboxy groups. Raubitschek (1961) in challenging the thesis that the increase in amino acids observed in substrates by the action of ringworm fungi is due to keratolysis, has suggested that they originate from fungal autolysis. This problem was further studied using electronmicroscopy (Mercer and Verma 1963), Polarised microscopy (Verma 1965) and fiuorescent microscopy techniques Verma 1966) It was found that T. mentagrophytes, peforate the human hair in vitro by a process of enzymatic digestion.

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